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Process efficiency and ventilation requirement in black soldier fly larvae composting of substrates with high water content

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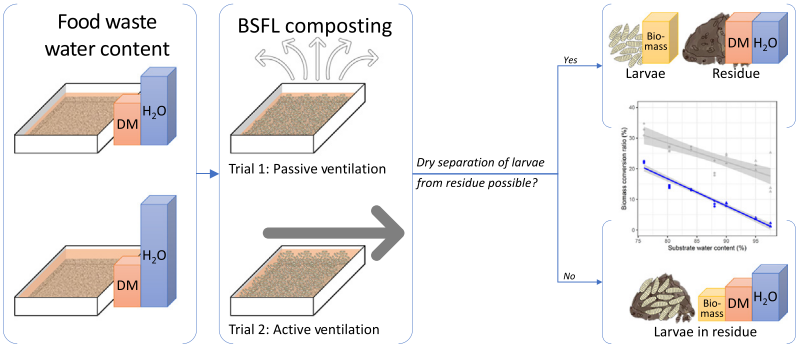
HIGHLIGHTS

- Impact of water content on fly larvae composting efficiency evaluated
- Higher water content reduced process efficiency.
- Possible to fly larvae treat substrates with water content 80–90%
- Ventilation requirement for attaining dry residue predicted
- Enables simple BSFL treatment of wet substrates such as veg & fruit peels

GRAPHICAL ABSTRACT



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ABSTRACT

In order to transition from a linear to a circular economy in the organic waste management sector, more of the elements in waste need to be recycled. Use of black soldier fly (*Hermetia illucens* L.; Diptera: Stratiomyidae) larvae (BSFL) for organic waste treatment has potential to harvest more complex molecules than conventional methods. Many organic waste substrates have high water content (>80%), but the impact on BSFL treatment efficiency of substrate water contents >80% is not known. This study evaluated the impact of high water content food waste on BSFL composting efficiency in terms of waste-to-biomass conversion ratio, material reduction, larval survival and the ventilation required for enabling dry separation of larvae from residue. In total, six water contents ranging from 76% to 97.5% were evaluated in two experimental trials. It was found that increasing water content reduced biomass conversion ratio and survival rate of the larvae, from 33.4% of volatile solids (VS) and 97.2% survival in 76% water to 17.5% of VS and 19.3% survival in 97.5% water. Furthermore, we found that the ventilation requirement for achieving dry separation of larvae from residue could be modelled by estimating the amount of water that would need to be removed, taking into account the water bound in the larvae, and knowing the specifics of the ventilation set-up of the modelled system. The findings could have implications on the waste management sector interested in implementing BSFL treatment, as the findings demonstrate that it is possible to treat wet substrates (such as fruit and vegetable wastes) without any pre-treatment other than grinding and attain an adequately dry residue for enabling dry separation of the larvae from the residue.

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1. Introduction

In 2015, the European Commission (EC) launched an action plan on waste handling and a circular economy in the European Union (EU), in which reusing the resources contained in waste is mentioned as one way to reduce dependence on new resources, and as an important step in transition from a linear to a circular economy model (European Commission, 2015). One of the focus areas in the action plan is food waste, and it specifically states that the markets will be stimulated to recover nutrients (European Commission, 2020). However, handling of biodegradable waste is costly and the products generated often have low economic value (Hogg et al., 2003).

A new waste management technique that has attracted considerable interest among researchers, the media, the public and waste handling entrepreneurs in recent years is fly larvae composting (Čičková et al., 2015). This is a robust and efficient biodegradable waste treatment that increases the value of the waste by utilising more complex molecules in the waste, such as amino acids and lipids, whereas current systems focus on simpler elements such as ammonia and methane (Lohri et al., 2017). The most commonly used fly species is black soldier fly (*Hermetia illucens* L.; Diptera: Stratiomyidae) and its polyphagous larvae (BSFL) have been demonstrated to feed on a large variety of decomposing organic matter, such as food waste (Surendra et al., 2016), human excreta (Banks et al., 2014; Lalander et al., 2013) and different animal manures (Myers et al., 2008; Sheppard et al., 1994; Xiao et al., 2018). The larval biomass comprises around 40% protein on a dry matter basis (Lalander et al., 2019), while the fat content and fatty acid profile depend on the substrate (Ewald et al., 2020; Meneguz et al., 2018). The primary use of the larval biomass is as a protein source in animal feed (Kroeckel et al., 2012), where it can replace some less sustainable alternatives such as soybean and fish meal (Costa et al., 2007; FAO, 2018). The fat fraction can be used for biodiesel production, or as a component in animal feed (Surendra et al., 2016). The treatment residues can be used as organic fertiliser (Setti et al., 2019) or as feedstock in the anaerobic digestion process (Lalander et al., 2018).

Interest in BSFL treatment of strictly vegetable-based substrates has increased in Europe in recent years, due to BSF being classified as production animals in the EU (Regulation (EC) 1069/2009). As they are production animals, and thus only allowed to be given strictly plant-based substrates, such as vegetable and fruit wastes. The water content of many of these globally available substrates are generally high; in an assessment of characteristics of Brazilian fruit and vegetable waste conducted over a year, Edwiges et al. (2018) found the mean water content to be 90.5%. In accordance with this, Garcia et al. (2005) found the water content of Spanish source separated household waste to be around 67%, while that of fruit and vegetable waste was 90%. Parra Paz et al. (2015) demonstrated that the several process parameters such as larval density and larval feeding rate had an impact on the overall process efficiency. Cheng et al. (2017) evaluated the impact of substrate water content on the larval survival and growth, and on the larvae-residue separation upon completed BSFL composting. They found that to enable dry separation of larvae from residue, the initial substrate water content could not be higher than 80%, as the water did not evaporate sufficiently during the BSFL composting (Cheng et al., 2017). The impact of water content on the process efficiency has to the best of our knowledge not been assessed. The water content of the majority of larvae feedstocks studied to date is typically around 70–80% (Lalander et al., 2019; Liu et al., 2018; Myers et al., 2008). In accordance with the findings of Cheng et al. (2017), Dortmans et al. (2017) stated in a step-by-step guide to BSFL composting that substrates with water content >80% must be de-watered before composting. A wet separation of the larvae from the treatment residue may still be required. Wet separation, is a more cumbersome and time consuming process, as compared to dry separation (Dortmans et al., 2017).

The purpose of this study was to evaluate the effect of substrate water content on BSFL composting efficiency of food waste and to evaluate the feasibility of using waste substrates with high water content (>80%) and to assess the ventilation requirement for attaining an adequately dry treatment residue that would allow for dry separation.

2. Materials and methods

2.1. Materials

For all BSFL composting experiments, household food waste (FW), collected and milled by Eskilstuna Strängnäs Energi och Miljö (Eskilstuna, Sweden), was used as feedstock. The average dry matter content was 22.5 ± 1.8% ($n = 6$), the volatile solids (VS) content was 88.4 ± 1.8% ($n = 6$) on a dry matter (DM) basis and the pH was within the range 4.2 (lowest) and 4.4 (highest) of the food waste used in all trials. The water content of the feedstock was adjusted for each set of experiments to the desired level of substrate DM by addition of water. Black soldier fly larvae (5 d old, >0.2 cm long) obtained from a BSF colony continuously running since 2015 at SLU (Uppsala, Sweden) were used in all treatments.

2.2. Experimental set-up

To study the impact of substrate water content on the efficiency of fly larvae composting, two different sets of experiments were conducted: in *Trial 1*, substrates with 76%, 84% and 88% water contents were investigated under *passive ventilation*, while high water content substrates of 90%, 95% and 97.5% water were investigated in *Trial 2* under *active ventilation*. The set-up between the trials differed, in response to the change in conditions with increasing substrate water content (Table 1). Results from the trials on the water removal capacity of the investigated system and process efficiency were used to model the ventilation requirement for attaining an adequately dry residue to allow for dry separation. Based on the findings of Cheng et al. (2017), it was assumed that the residue had to have a DM content of 50% in order to allow for dry separation.

Trial 1 was conducted in small containers with surface area of 357 cm^2 (Smartstore classic 2, 21 cm × 17 cm × 11 cm) and 700 larvae (lv) in each. In Trial 2, the experiments were conducted in larger

Table 1
Daily feeding dose of wet weight and volatile solids (kg WW and VS m^{-2}), total substrate depth (cm), larval feeding dose (g VS lv^{-1}), treatment area (cm^2) and larvae density (lv cm^{-2}) in the seven treatments.

Substrate water content	Trial	Daily WW feeding dose (kg WW m^{-2} d^{-1})	Daily VS feeding dose (kg VS m^{-2} d^{-1})	Total substrate depth (cm)	Larval feeding dose (g VS lv^{-1})	Treatment area (cm^2)	Larvae density (lv cm^{-2})
76% water	1	2.3	0.5	3.2	0.35	357	2
84% water	1	3.3	0.5	4.6	0.35	357	2
88% water	1	4.3	0.5	6.0	0.35	357	2
90% water	2	1.2	0.1	2.0	0.09	2400	2
95% water	2	2.4	0.1	4.0	0.09	2400	2
97.5% water	2	4.8	0.1	8.0	0.09	2400	2
80% water (C)	C	3.1	0.4	6.0	0.17	2400	6

containers with surface area of 2500 cm^2 (60 cm × 40 cm × 12 cm) and 5000 larvae. The larger containers were used to increase the treatment surface and allow higher evaporation rates when testing wetter substrates. The larval VS feeding dose was 0.35 g VS lv^{-1} in Trial 1 but was reduced to 0.08 g VS lv^{-1} in Trial 2 to avoid wet substrate build-up (substrate depth). The substrate depth should not exceed 5 cm according to Dortmans et al. (2017) as the larvae otherwise will not be able to process the material at the bottom of the container. In some of the treatments, the total substrate depth would be >5 cm (Table 1); however, the substrate was provided in three feeding occasions in Trial 1, and daily feedings in Trial 2, and thus it was assumed that the total material depth would not exceed 5 cm at any time in the treatment. The larval density was kept the same in both trials (2 lv cm^{-2}). The duration of Trials 1 and 2 was 15 and 14 days, respectively. A control treatment (C, 80% water) was also established, using parameters based on a protocol developed at our department described in detail in Johannesdottir (2017), and was used to verify whether the findings for wetter substrates applied over a broader set of parameters.

2.3. Experimental execution

In Trial 1, the total substrate in each treatment was split into three parts and fed to larvae on days 1, 5 and 8. On day 1, the 700 young larvae and the first food waste portion were placed in the treatment container. The containers were kept in an open room and were covered with lids that had a rectangular meshed opening of about 5 cm × 3 cm. Each treatment in Trial 1 was conducted in triplicate.

In Trial 2, the larvae were fed five times per week (no feeding during weekends). On day 1, the daily feeding portion and 5000 young larvae were placed in the treatment boxes. The larvae were added with a small portion of treatment residue (~5 g) as support material for the larvae in the wet substrate. The open boxes were placed in a ventilated reactor chamber (3.2 m^3). Ventilation was provided by a set of four channel fans (Rörfläkt ø100 mm, Biltema, Sweden) with maximum capacity of 107 m^3 h^{-1} each, blowing the air downwards through openings in the roof of the reactor chamber. Two ventilation fans (Ventilationsfläkt 100 mm, Biltema, Sweden), with maximum capacity of 187 m^3 h^{-1} each, were connected to two channel pipes at the bottom of the reactor chamber and removed the air at roughly the same rate as it was supplied. The ventilated reactor chamber was also equipped with two sets of combined air temperature and humidity sensors (AM2302/DHT22, Adafruit, USA), which sampled the air in each ventilation inlet and outlet at 5 min intervals. Data were recorded using a Raspberry Pi (V. 3B, Raspberry Pi Foundation, UK) data logger. The daily feeding supplied on Monday-Thursday was constant (Table 1), while on Fridays a triple daily feed amount was applied to compensate for the lack of feeding during the weekends. The experiments in Trial 2 were repeated in three runs, conducted in singlets.

In the Control process, as in the Trial 1, the total feedstock was split into three portions, which were added to the boxes on days 1, 5 and 8. The control had higher larval density (6 lv cm^{-2}) in order to achieve an intermediate larval feeding dose and comparable substrate depth to that in the two trials (Table 1). The Control process was run in triplicate, in the same boxes and ventilation conditions as in Trial 2.

2.4. Sampling and analysis

The young larvae used for the experiments in Trial 1 were counted manually. For Trial 2, the numbers of larvae were estimated based on average larval weight, which was measured by weighing and counting three sub-samples of roughly 100–200 larvae. All grown larvae at the end of the experiments in Trial 1 were manually picked out of the treatment residues and weighed to measure total larval biomass. In Trial 2, the grown larvae were sieved when possible (dry residue). In cases when dry separation was not possible (too wet residue), the larvae were picked out manually. In the dry separation, the larvae and residue were placed on a mesh covered table (mesh size 50 mm) and the table was shaken automatically. The residue falls through the mesh, while the larvae remain on the mesh. The total larval biomass was weighed and the total final number of larvae was determined by dividing total larval biomass by average individual larvae weight, established by enumerating and weighing a sample of 100 randomly selected larvae. The grown larvae samples used for determination of DM and VS content were killed by storage at −20 °C for 24 h prior to measurements. The DM and VS contents were determined and the weight of the treatment residues was measured for each replicate in Trial 1. In all treatments in Trial 2, only residue wet weight was recorded.

The DM content in samples of initial substrate, treatment residues and grown larvae was determined gravimetrically. The wet samples were weighed in aluminium cups and dried at 70 °C to stable weight, but for a minimum of 48 h, and the change in sample weight recorded was taken to represent the water loss. A drying temperature of 70 °C was selected to avoid loss of organics in the samples (Vahlberg et al., 2013). Volatile solids content was measured by gravimetric ash content determination after combusting the dry samples in an oven at 250 °C for 1 h and at 550 °C for 4 h (ISO 18122:2015).

2.5. Calculations

Bioconversion ratio was calculated on the basis of wet weight [WW], dry matter [DM] and volatile solids [VS] ($BCR_{[WW,\ DM,\ VS]}$, respectively):

$$BCR_{[WW,DM,VS]} = \frac{m[WW, DM, VS]_{res}}{m[WW, DM, VS]_{sub}} \quad (1)$$

where m[WW, DM, VS]$_{res}$ and m[WW, DM, VS]$_{sub}$ is total wet weight, dry weight and total volatile solids weight of residues (*res*) and incoming substrate (*sub*), respectively.

The required water removal per kg WW (mH_2O_{rem}) to achieve a DM of 50% (Res_{DM} = 50%) was calculated as:

$$mH_2O_{rem} = \left(mH_2O_{sub} - \left(\frac{mDM_{sub} \times Red_{DM}}{50\%}\right) - (mDM_{sub} \times BCR_{DM} \times mH_2O_{lv})\right) \times \frac{1}{mWW_{sub}} \quad (2)$$

where mH_2O_{sub} and mDM_{sub} are total water and dry matter weight of the incoming substrate (g), Red_{DM} and BCR_{DM} are material reduction and biomass conversion ratio (%) in the process on a dry matter basis, mH_2O_{lv} is total water weight in the larvae produced (g) and mWW_{sub} is total wet weight (g) of incoming substrate.

The average amount of water removed per kg air exchanged ($\overline{m}H_2O_{air}$, g kg^{-1}) during the three sets of experiments in Trial 2 was calculated based on the difference in measured temperature and relative humidity (RH) between the incoming and outgoing air as:

$$\overline{m}H_2O_{air} = \frac{1}{n}\sum_{i=1}^{n}(RH_{out,i} \times X(T_{out})_i - RH_{in,i} \times X(T_{in})_i) \quad (3)$$

where $X(T_{out/in})_i$ is the water vapour holding capacity of air at specific temperatures of outlet and inlet air for each set of measurements (i) for n number of sets. The saturation vapour capacity in dry air was calculated using standard equations in which the saturation pressure of vapour at temperature T (P_T) was calculated using Buck's equation (Buck, 1996).

The mass of air ($mAir_{rem}$) required to remove mH_2O_{rem} was calculated as:

$$mAir_{rem} = \frac{mH_2O_{rem}}{\overline{m}H_2O_{air}} \quad (4)$$

while Q_{rem}, the required ventilation volume of air per hour (m^3 h^{-1}),

was calculated as:

$$Q_{rem} = \frac{mAir_{rem}}{\rho_{air,\overline{T}}} \times t_{exp} \quad (5)$$

where $\rho_{air,\overline{T}}$ (kg m^{-3}) is the density of air at the average temperature of the inlet air over the three sets of measurements ($\overline{T}$) and t_{exp} is duration of the experiment (h).

2.6. Statistical analysis

Analysis of variance (ANOVA) with 95% confidence interval was performed to establish statistically significant differences between the outcomes of the treatments. When a statistical difference was found, Tukey's honestly significant difference (HSD) post hoc test with 95% confidence interval was performed in order to identify significant differences between the treatments. A generalised linear model with 95% confidence level was used to perform the regression analyses. The model residuals were verified for normality. Larval survival rate was found not to be normally distributed in the ANOVA and was converted into $\log_{10}$ mortality rate.

The data were analysed in R (RStudio Team, 2016) and all graphical representations were created using the R-package ggplot2 (Wickham, 2016).

3. Results and Discussion

3.1. Process efficiency

A significant reduction in BCR was observed with increasing substrate water content, on both a WW and VS basis (Table 2), with a negative correlation ($R^2 = 0.96$, $p < 0.001$) between BCR on a WW basis and substrate water content (Fig. 1a, Table 3). There was also a significant correlation ($p < 0.001$) between BCR on a DM basis and substrate water content, but with a weaker model fit ($R^2 = 0.64$). A negative correlation ($p < 0.05$) between material reduction and substrate water content on a DM basis was found in Trial 1 (Fig. 1b), but no correlation was found when including the Control. However, when also including the VS dose fed to the larvae (g VS larva^{-1}), there was a significant correlation also including the Control ($R^2 = 0.94$, $p < 0.001$) (Fig. S1).

The strong impact of substrate water content on BCR demonstrated that biomass conversion efficiency was reduced even if the same amount of nutrients was supplied. Although the VS dose fed to larvae also had some impact on BCR, the substrate water content had an overriding impact (Model 3 BCR_{DM} in Table 3), such that at a given VS dose per larva, substrate water content defined the BCR (Fig. 1a). The substrate water content used for fly larvae composting in previous studies has been within the range 65–85% (Banks et al., 2014; Cheng et al., 2017; Li et al., 2011; Meneguz et al., 2018). The substrate water contents evaluated in this study were within the range 76–97.5%, and it was found that a water content of 76–84% gave similar or higher BCR than in the Control (water content 80%) on a VS basis (around 30% of VS) (Table 2). On a WW basis, BCR almost doubled from water content 84% to 76%. Diener et al. (2011) found BCR of 11% on a WW basis for BSFL composting of food waste with a water content of 76%, which is similar to the BCR value found for substrates with a water content of 84% in this study (13.2%) (Table 2). However, unlike the substrate in this study, the waste used by Diener et al. (2011) was not homogenised prior to BSFL composting, which could explain the lower conversion efficiency. Win et al. (2018), on the other hand, obtained a BCR of 18.4% on a WW basis for BSFL composting of food waste with water content of 75.5%. This is comparable to the BCR_{WW} (22.2%) found in this study at a substrate water content of 76% (Table 2). One reason for the lower BCR in Win et al. (2018) is likely the lower larval feeding dose, 0.63 g WW larva^{-1} compared with 1.7 g WW larva^{-1} in this study (Table 1). Parra Paz et al. (2015) demonstrated that a higher larval feeding rate results in higher relative growth rate.

A trend for lower material reduction with increasing water content was found in Trial 1 (Table 2). In contrast to this trend, a higher material reduction was found for the Control, which had a higher water content (80%) than the 76% treatment (Table 2). Although BCR was not significantly different between these two treatments, the material reduction was lower in the 76% treatment. This difference could be partly attributable to the higher larval VS feeding dose in the 76% treatment (0.35 g VS larva^{-1}) than in the Control (0.17 g VS larva^{-1}), thus leading to more VS being available for larvae to begin with. This agrees with the relationship reported by Parra Paz et al. (2015) of lower material reduction at higher feeding rate but with higher larval density, resulting in a lower material reduction at the same feeding rate. The larval density in our Control was 6 lv cm^{-2}, while it was 2 lv cm^{-2} in the 76% treatment in Trial 1 (Table 1). Based on the relationship demonstrated by Parra Paz et al. (2015), if the feeding rate in the Control had been as high as in Trial 1, the material reduction in the Control might have been even lower than that found in the 76% treatment. Another factor differing between the 76% treatment and the Control was that the latter was performed under active ventilation, so the water content of the material would have been reduced more rapidly, which could favour material reduction. Lundgren (2019) demonstrated higher DM reduction rate and lower BCR when BSFL composting was performed in ventilated open boxes in contrast to boxes with closed perforated lids, while other process conditions were similar to those used in the present study. The higher reduction rate in open boxes was attributed to increased microbial respiration associated with increased ventilation. As in conventional composting, substrates with high water content result in reduced aerobic microbial activity due to more limited aeration, and thus also achieve a smaller material reduction (Ermolaev et al., 2019).

3.2. Ventilation

Substrate water content and type of ventilation significantly affected the amount of water removed in the process (Table 4). Higher water content in the inflow substrate led to higher water content in the treatment residues. The active ventilation implemented for the wetter

Table 2
Biomass conversion ratio (BCR) and material reduction on an initial (init.) wet weight (WW) and volatile solids (VS) basis, larval survival and mean larval weight in the experiments in Trials 1 and 2 and the Control (C). Mean ± standard deviation ($n = 3$). Different letters within columns indicate significant difference ($p < 0.05$).

Substrate water content	BCR		Material reduction		Larval survival*	Larval weight
	% init. WW	% init. VS	% init. WW	% init. VS	% init.	mg larva^{-1}
76% water	22.2 ± 0.3	33.4 ± 2.1^{a}	39.6 ± 1.8^{a}	48.1 ± 1.5^{a}	97.2 ± 0.9^{a}	373 ± 2^{a}
84% water	13.2 ± 0.2^{a}	27.1 ± 0.7^{a,b}	24.9 ± 0.4^{a}	43.6 ± 2.9^{a}	94.3 ± 0.9^{a}	342 ± 7^{a,b}
88% water	8.5 ± 0.9^{b}	20.4 ± 2.7^{b,c}	31.3 ± 1.6^{a}	35.2 ± 2.9^{b}	91.2 ± 1.2^{a}	305 ± 36^{b,f}
90% water	8.4 ± 0.6^{b}	24.2 ± 1.6^{c,b}	95.0 ± 0.6^{b,c}		78.9 ± 18.3^{a,b}	104 ± 21^{c}
95% water	3.8 ± 0.3	21.6 ± 1.5^{c,b}	89.7 ± 5.7^{b}		56.6 ± 11.9^{b}	130 ± 12^{c}
97.5% water	1.5 ± 0.6	17.5 ± 7.2^{c}	76.6 ± 15.6^{c}		19.3 ± 15.9^{c}	236 ± 46^{d,f}
80% water (C)	14.3 ± 0.4^{a}	27.4 ± 1.1^{a,b}	79.9 ± 2.0^{b,c}	69.2 ± 2.0^{c}	62.6 ± 2.8^{b}	214 ± 29^{d}

* Statistical differences based on results of ANOVA for $\log_{10}$ mortality rate.

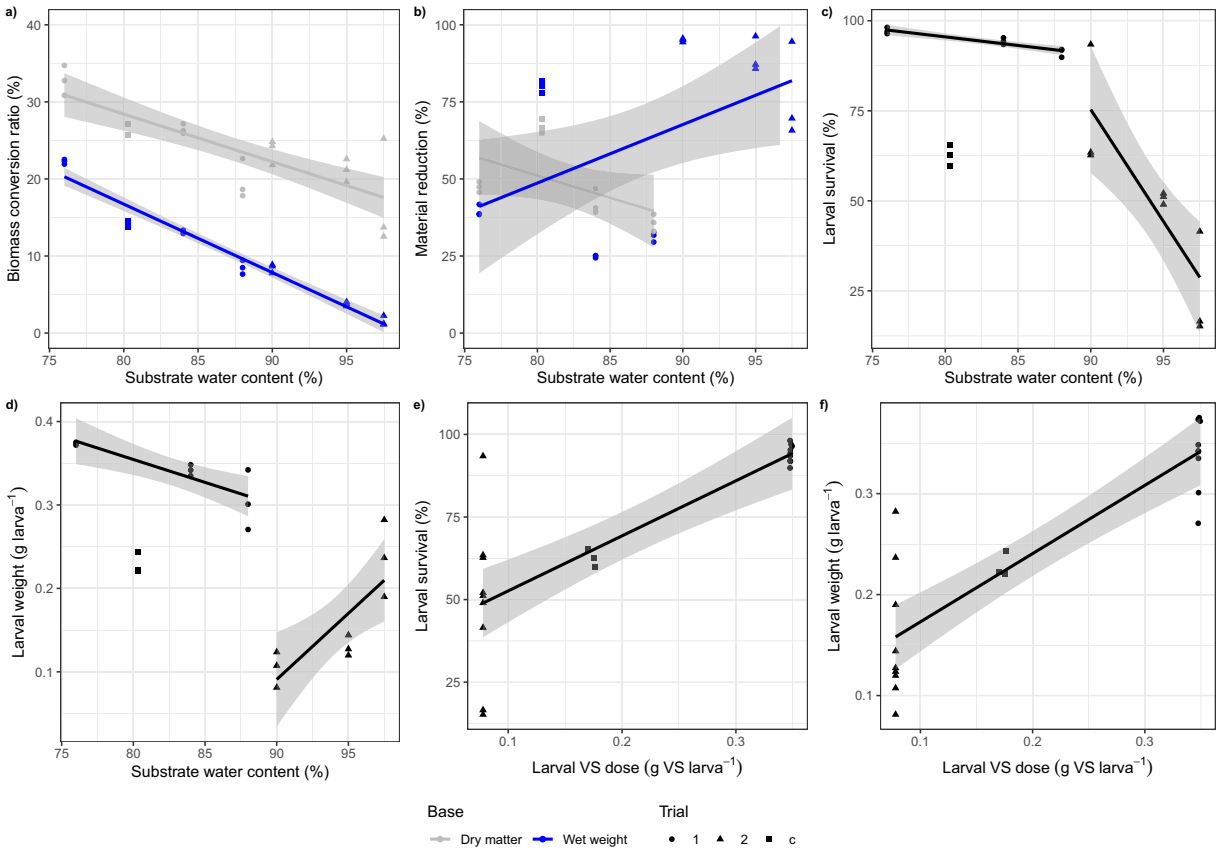

Fig. 1. Effects of substrate water content (%) on a) substrate-to-biomass conversion ratio (%), b) material reduction on a dry matter basis (dark grey line) and a wet weight basis (light grey line), c) larval survival and d) larval weight, and effects of larval volatile solids (VS) feeding dose on e) larval survival (%) and f) larval weight (g larva^{-1}) in Trial 1 (●), Trial 2 (▲) and Control (■). For all models, the grey zones represent the model fit with 95% confidence level.

substrates (Trial 2) resulted in a larger variation in residue DM than seen with passive ventilation (Trial 1), and resulted in greater variation in the amount of water removed with increasing substrate water content. The required water removal ($m\mathrm{H_2O}_{rem}$) was estimated based on the assumption that treatment residues needed a DM content of 50% for achieving dry separation. The requirement was found to increase proportionally to the increase in initial substrate water content. The amount of water removed was not sufficient except in the treatment with 90% substrate water content, where more water than required was removed, resulting in a residue DM content of almost 60% (Table 4).

In Trial 2, 74–85% of the WW reduction was due to water evaporation (Table 4). On comparing total WW reduction (Table 2), it was found that water removal accounted for 86–97% of the total reduction. Since the experiments with water content 90–97.5% were run in parallel in the reactor chamber, similar ventilation ($m^3\ h^{-1}\ kg^{-1}$ WW) was provided, even though the theoretical ventilation requirements were not

Table 3
Regression model and equations for different response variables: the rate constant (k) representing rate of change in evaluated response value and R^2 the fit of the model.

	Equation	Response variable	Model parameters		
			R^2 adjusted	k_1	k_2
Model 1 BCR $_{WW}$	$\mathrm{BCR}_{WW} = \mathrm{m} + \mathrm{m}k_1\ \%\mathrm{H_2O}_{sub}$	BCR_{WW}	0.96	-0.89^{***}	
Model 2 BCR $_{WW}$	$\mathrm{BCR}_{WW} = \mathrm{m} + k_1$ lv.VS.dose	BCR_{WW}	0.45	35.5^{***}	
Model 1 BCR_{DM}	$\mathrm{BCR}_{DM} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub}$	BCR_{DM}	0.64	-0.62^{***}	
Model 2 BCR_{DM}	$\mathrm{BCR}_{DM} = \mathrm{m} + k_1$ lv.VS.dose	BCR_{DM}	0.21	19.8^{*}	
Model 3 BCR_{DM}	$\mathrm{BCR}_{DM} = \mathrm{m} + k_2\ \%\mathrm{H_2O}_{sub} + k_1$ lv.VS.dose	BCR_{DM}	0.65	-0.73^{***}	$9.17^{■}$
Model Red	$\mathrm{Red} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub}$	Mat. red_{WW}	0.20	1.90^{*}	
Model 1 Red_{DM}	$\mathrm{Red}_{DM} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub}$	Mat. red_{DM}	0.29	$-1.44^{■}$	
Model 1.b $\mathrm{Red}_{DM[Trial\ 1]}$	$\mathrm{Red}_{DM} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub}$	Mat. red_{DM} [Trial 1]	0.74	-0.92^{**}	
Model 2 Red_{DM}	$\mathrm{Red}_{DM} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub} + k_2$ lv.VS.dose	Mat. red_{DM}	0.94	-0.93^{**}	-132.1^{***}
Model $\mathrm{SR}_{Trial\ 1}$	$\mathrm{SR}_{Trial\ 1} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub}$	Larval survival [Trial 1]	0.86	-0.48^{***}	
Model $\mathrm{SR}_{Trial\ 2}$	$\mathrm{SR}_{Trial\ 2} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub}$	Larval survival [Trial 2]	0.69	-6.2^{**}	
Model SR 1	$\mathrm{SR} = \mathrm{m} + k_1$ lv.VS.dose	Larval survival	0.66	167^{***}	
Model SR 2	$\mathrm{SR} = \mathrm{m} + k_1$ lv.VS.dose $+ k_2\ \%\mathrm{H_2O}_{sub}$	Larval survival	0.67	125^{**}	$-1.0^{■}$
Model $\mathrm{LV}_{WW\ [Trial\ 1]}$	$\mathrm{LV}_{WW\ [Trial\ 1]} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub}$	Larval weight [Trial 1]	0.64	-0.005^{**}	
Model $\mathrm{LV}_{WW\ [Trial\ 2]}$	$\mathrm{LV}_{WW\ [Trial\ 2]} = \mathrm{m} + k_1\ \%\mathrm{H_2O}_{sub}$	Larval weight [Trial 2]	0.59	0.016^{*}	
Model LV_{WW}	$\mathrm{LV}_{WW} = \mathrm{m} + k_1$ lv.VS.dose	Larval weight	0.76	0.68^{***}	
Model LV_{WW}	$\mathrm{LV}_{WW} = \mathrm{m} + k_1$ lv.VS.dose $+ k_2\ \%\mathrm{H_2O}_{sub}$	Larval weight	0.75	0.68^{***}	$2 \times 10^{-5■}$

Significance level of model probability value: $p < 0.001^{***}$, $p < 0.01^{**}$, $p < 0.05^{*}$.
$\%\mathrm{H_2O}_{sub}$ = substrate water content; lv.VS.dose = larval VS feeding dose. For other abbreviations, see text.

Table 4
Effects of ventilation type and amount of ventilation provided ($m^3\ h^{-1}\ kg^{-1}$ wet weight (WW)) on the dry matter content (DM) of treatment residues and on the average amount of water removed (g kg^{-1} WW), and estimated required water removal rate and ventilation requirement to reach a treatment residue DM content of 50% ($m^3\ h^{-1}\ kg^{-1}$ WW). Mean ± standard deviation ($n = 3$). Different letters within columns indicate significant difference ($p < 0.05$).

	Treatment residue DM	Water removed	Ventilation provided	Required water removal[2]	Estimated ventilation requirement[2,3]
	% WW	g kg^{-1} WW	$m^3\ h^{-1}\ kg^{-1}$ WW	g kg^{-1} WW	$m^3\ h^{-1}\ kg^{-1}$ WW
76% water	21.0 ± 0.0	139.3 ± 16.1		491 ± 2	0.86 ± 0.003
84% water	12.3 ± 0.8[a]	91.4 ± 5.7		655 ± 2[a]	1.15 ± 0.004[a]
88% water	11.2 ± 0.2[a]	208.4 ± 7.0		743 ± 6	1.31 ± 0.011
90% water	59.4 ± 6.9[1]	818.7 ± 7.1[1]	1.37 ± 0.49	810 ± 4	1.42 ± 0.007
95% water	23.7 ± 19.6[1]	837.2 ± 57.0[1]	1.39 ± 0.48	906 ± 2	1.59 ± 0.003
97.5% water	7.4 ± 8.0[1]	739.5 ± 159.3[1]	1.22 ± 0.43	955 ± 6	1.68 ± 0.008
80% water (C)	32.7 ± 5.4	576.7 ± 0.05		648 ± 2[a]	1.14 ± 0.004[a]

[1] Calculated value based on DM reduction obtained in Model 2 Red_{DM} in Table 3, values not included in statistical analysis.
[2] For reaching treatment residue DM content of 50% (Eq. (2)).
[3] Calculated using Eqs. (3)–(5).

the same (Table 4). In Trial 1, no active ventilation was applied and thus the amount of water removal was considerably smaller than that required to reach a DM of 50% (Table 4). BCR, material reduction and larval survival were affected by substrate water content (Table 2).

3.3. Impact on larval survival and weight

Larval survival was found to be negatively impacted by substrate water content in both trials ($R^2 = 0.86$ in Trial 1, $R^2 = 0.69$ in Trial 2) (Fig. 1c, Table 3). A negative correlation between individual larval weight and substrate water content was found for Trial 1 ($R^2 = 0.64$) and a marginally weaker positive correlation was found for Trial 2 ($R^2 = 0.59$) (Fig. 1d, Table 3). Water content was found to have an impact on larval weight (either positive or negative), while larval VS dose had a positive correlation ($R^2 = 0.76$) (Table 3). In contrast, when all data (including the Control) was included, larval feeding dose was found to have a significant impact on the survival and weight of the larvae, with higher survival and weight being achieved with higher feeding dose (Fig. 1e-f; Table 3). Cheng et al. (2017) found BSFL survival rates of 95–97% when treating pre- and post-consumer food waste, with neither survival nor larval weight being impacted by water contents in the range 70–80%. Similar findings were made in this study, where the average survival was around 97% at 76% substrate water content, and the larval weight not significantly different at 76% and 84% (Table 2). However, at higher water contents, both the survival and larval weight were impacted. The high water content of the substrate made it lose its structure. Structure is crucial to aerate the substrate and allow the larvae to actively move through it while maintaining unobstructed respiration through their spiracles, located at the anterior and posterior ends of the body (Barros et al., 2019). In addition, separation of liquid phase and suspended solids in the substrate was observed (Fig. S2a). Within the first few days (24–72 h) after introduction to the food waste, the young larvae aggregated at the sides of the treatment container or at the surface of the substrate. This was most likely due to them feeding on the more easily ingested dissolved organics in the liquid phase. As the water evaporated (more rapidly in Trial 2, cf. Table 4), the larvae that were feeding at the sides of the treatment container got stuck or dried out and the dissolved organic matter became concentrated and sticky (Fig. S2b). High substrate water content and poor structure could also have caused problems for the larvae in later instars. As larvae grow and develop through instars, their weight increases and their morphology changes, with their ventral and dorsal setae becoming shorter (Barros et al., 2019). As the material depth increased in the treatment containers (due to continuous addition of high water content substrate), the larvae increasingly migrated into the material in order to allow their posterior spiracles to breathe through the water surface. This left the larva floating vertically with their thorax pointing downwards. In this position, it is likely that small disruptions (other larvae disturbing, shaking of the treatment container, new feeding batch being added) would cause the spiracles

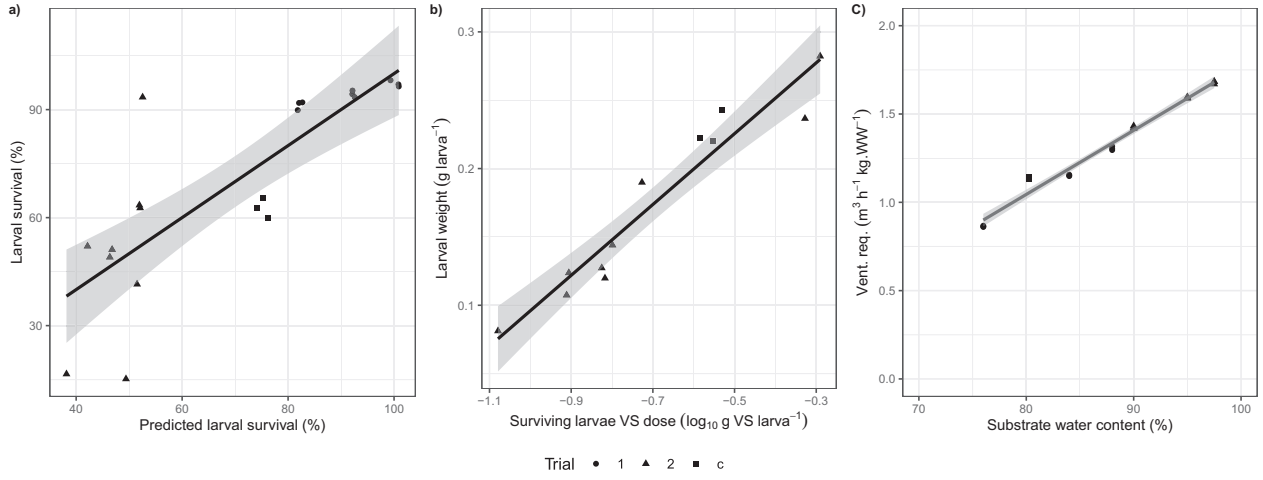

Fig. 2. Correlations between: a) measured and predicted larval survival (%), calculated using the model function $y = 202–0.045mH_2O_{rem} – 1.3\%H_2O_{sub}$, in which the relationship of larval survival to total mass of water removed in the treatment (mH_2O_{rem}) and substrate water content ($\%H_2O_{sub}$) were considered (*adjusted* $R^2 = 0.64$); b) larval weight (g $larva^{-1}$) and logarithmised larval VS dose (log_{10} g VS $larva^{-1}$) based on only surviving larvae (*adjusted* $R^2 = 0.92$); c) estimated ventilation requirement during BSFL composting to obtain treatment residues with 50% DM (estimated using the model function $y = -1.86 + 0.036k$) and substrate water content (*adjusted* $R^2 = 0.98$). The grey zone represents the model fit with 95% confidence level.

to lose contact with the water surface, which would leave the larvae unable to breathe and thus drown. Another likely reason for the high larvae mortality in Trial 2 was the stronger active ventilation implemented to dry the very wet substrates (>90%). Such ventilation was necessary to limit substrate depth build-up and to achieve the desired residue DM (50%). The ventilation efficiently removed water and, although it was not sufficient to attain a residue DM content of 50%, it did prevented excess increase in material depth. In the wettest treatment, the material depth at the end of the experiment was <2 cm in all replicates. Plotting actual larval survival against predicted model values of larval survival in which both the substrate water content and the total water removal (g kg^{-1} WW) were taken into account gave a fairly good fit ($p < 0.05$, R^2-adjusted = 0.64) (Fig. 2a). This shows that the wetter the substrate and the higher the water removal rate, the lower the larval survival. As the survival was reduced, the VS per remaining larva increased. Regression analysis of larval weight against the logarithmised VS dose of the surviving larvae revealed a significant positive correlation ($p < 0.05$, R^2-adjusted = 0.92) for Trial 2 and the Control, i.e. the higher the VS dose, the higher the larval weight (Fig. 2).

3.4. Estimating ventilation requirements

The fly larvae composting process itself had a very small influence on the final residue DM, which was mostly affected by the ventilation, in particular for substrate with higher water content. Normally, in order to estimate the amount of ventilation required, information on the water content of incoming material and outgoing products from the system is needed. In the present study, we developed a set of models (Table 3) that allow estimation of BCR (Fig. 1a) and material reduction (Table 2) based on the water content of incoming food waste and larval VS feeding dose. On deciding the desired residue DM, we combined these models to estimate the required water removal (Eq. (2)). Knowing the specifics of the ventilation set-up used (temperature and RH), it is possible to predict the ventilation demand for treatment of food waste in a BSFL composting system in an enclosed reactor chamber (Eqs. (3)–(5)), and the ventilation requirements per kg initial substrate WW were calculated. A linear increase in ventilation demand with increasing water content was found, with the treatment aim to achieve sufficient water removal to obtain residues with 50% DM (Fig. 2c). Combination of the models predicting required ventilation based on substrate properties was confirmed to accurately predict the moisture content of the residues as measured in the Control. This has major implications for large-scale BSFL composting systems where, after necessary adjustments, it would be possible to regulate ventilation to a required level based on properties of the incoming substrate in order to achieve a residue moisture level suitable for dry separation of larvae.

3.5. Applicability

The ventilation requirement was very high for substrates with water content >90%. Treating these substrates required additional work (daily feedings), had a low capacity (0.1 kg VS m^{-1} d^{-1} compared with 0.5 kg VS m^{-1} d^{-1} for substrates with water content <90%) and had low process efficiency. Increasing the substrate water content from 76% to 97.5% doubled the ventilation demand per kg of substrate WW treated. These results suggest that it would not be feasible to fly larvae compost substrates with water content above 90%, mainly due to the large space, energy and workload requirements and small returns in terms of products generated. An alternative would be inclusion of a pre-treatment water removal step, but that has the disadvantage of generating a wastewater stream that requires further treatment and removing easily available, readily soluble nutrients in the separated water. However, in contrast to conclusions of Cheng et al. (2017), we believe that adequate ventilation enables BSFL treatment of substrates with water content 80–90%. This opens up for the possibility of BSFL treating wet substrates such as vegetable and fruit wastes, without the implications of wet separation described by Dortmans et al. (2017) for BSFL treatment of fruit waste.

4. Conclusions

It was found that increased water content resulted in reduced biomass conversion ratio and material reduction. Larval survival decreased with increasing water content, while larval weight decreased with increasing water content when the survival was high but increased with decreasing survival rate. A model was developed for predicting the ventilation requirement per kg of food waste wet weight in relation to substrate water content that could predict the required ventilation needed for attaining a residue adequately dry for dry separation of larvae from residue. It was found that it is possible to BSFL treat substrates of water content 80–90% by implementing ventilation, while very wet substrates with water content over 90% were deemed to not be suitable to BSFL compost even with active ventilation. These findings could have implications on the waste management sector interested in the implementation of fly larvae treatment of fruits and vegetable wastes, as BSFL composting of wet substrates would be simpler if a dry separation of larvae from residue is possible upon completed treatment, rendering this treatment option viable for a wider range of substrates.

CRediT authorship contribution statement

Cecilia Lalander: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization, Supervision. **Evgheni Ermolaev:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing. **Viktoria Wiklicky:** Investigation, Writing - review & editing. **Björn Vinnerås:** Conceptualization, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138968.

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